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Effect of calcium on rheological properties of *Abelmoschus esculentus (okra)* pod polysaccharide and its application in *Annona squamosa*

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Abstract

The fruit softening seriously affected the quality of *Annona squamosa*, and the edible coating combined with exogenous calcium had a positive effect on inhibiting the softening of *A. squamosa*. In this paper, the rheological properties of C-AeP-P solution and the effect of Ca^{2+} on the rheological properties of the solution were studied. Based on this, the indicators of fruit hardness, freshness and plumpness were used to monitor the softening effect of *A. squamosa*. The results show that: C-AeP-P solution showed non-Newtonian shear-thinning behavior over the range of 0.2-1.0%. With the increase of C-AeP-P concentration, the apparent viscosity and solids like properties increased. After the addition of Ca^{2+}, C-AeP-P had apparent differences on apparent viscosity. The G′ and G″ of C-AeP-P were increased with increasing oscillation frequency. C-AeP-P and C-AeP-P+Ca^{2+} treatment can slow down rapidly decline of fruit hardness, increase of soluble solid content, loss of water, change peel color and regulate the expression of key enzyme genes *AI* and *Ni*. It was concluded that the coating treatment of C-AeP-P alleviates the adverse effects of quality changes during storage, so as to improve the quality and prolong the shelf life of *A. squamosa*.

Keywords: *Abelmoschus esculentus (okra)*; polysaccharide; rheological properties; post-harvest treatment.

Practical Application: *Okra* pod polysaccharide treatment was developed into a new method for *Annona squamosa* fruit storage and preservation.

1 Introduction

Annona squamosa (*A. squamosa*) is a popular tropical fruit which has rich nutrition (Ren et al., 2019). The softening, chilling damage and browning of *A. squamosa* fruit during storage not only restricted the reduction of its economic value, but also caused the reduction of its nutritional value. Coating (Nie et al., 2020), air conditioning (Khodifad et al., 2018) and irradiation (Chouksey et al., 2013) are often used to solve the problems of fruit softening and extending the shelf life of fruits. However, coating preservation technology is widely used in fruits and vegetables with high moisture content because of its simplicity, convenience and low cost (Ali et al., 2020). Edible coatings with good oxygen isolation and antibacterial properties are used as a common material in coating preservation. Polysaccharides are long and complex chain carbohydrates with different functional and biological activities due to differences in structural characteristics (Li et al., 2022). The functional activity of coating film is one of the many activities of polysaccharides. In recent years, polysaccharide solution has become one of the ideal materials for edible coatings because of its rheological, thermal and degradable properties (Han et al., 2020; Ma et al., 2021; Zhong et al., 2020). In addition, exogenous calcium also plays an active role in the preservation of fruits and vegetables (such as participating in the composition of cell wall structure, affecting plant energy metabolism, regulating the damage of reactive oxygen species to postharvest fruits and vegetables to affect plant cell signal transduction, etc.). However, there are few reports on the synergistic effect of polysaccharides and exogenous calcium on the preservation of cherimoya. The results of this study are a new attempt to enrich the post-harvest storage and fresh-keeping solutions of *A. squamosa*.

The research shows that one of the key factors for polysaccharide to become an ideal edible coating is whether has well viscosity and the formation of gel; Meanwhile, viscosity and gel properties are closely related to rheological parameters. Among the many factors that affect the rheological properties of polysaccharide solution, the role of metal ions is a key factor (Geng et al., 2022; Sucheta et al., 2019); Of course, this effect also provides the possibility for the combination of exogenous calcium and polysaccharides to be applied to the preservation of *A. squamosa*. On the other hand, our team has found the key enzyme genes in the softening process of *A. squamosa* in previous experiments, which also provides technical support for the study of the combined effect of exogenous calcium and polysaccharides on the preservation of *A. squamosa* from the genetic level.

In this paper, the rheological properties of polysaccharide extracted from *Abelmoschus esculentus (okra)* pod (C-AeP-P) solution and the effect of calcium salt ions (Ca^{2+}) on the rheological properties of the solution are studied. On this basis,

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the appropriate concentration of C-AeP-P combined with Ca^{2+} coating was selected to treat *A. squamosa*; The coating effect was discussed by monitoring hardness, soluble solid content, weight loss rate, color and gene expression of key enzymes of glucose metabolism. This provides a new reference scheme for the solution of fruit softening control, extending shelf life and reducing the decline of post-harvest quality.

2 Materials and methods

2.1 Plants materials and reagents

Fresh *Abelmoschus esculentus (okra)* were harvested from Shandong, China. *A. squamosa* is purchased from a fruit and vegetable wholesale base in Guangdong, China. All the chemicals were used of analytical grade. R2070 plant RNA small extraction kit (Beijing solebao Technology Co., Ltd., China); G3330 RT first stand cDNA synthesis Kit (Wuhan saiweier Biotechnology Co., Ltd., China); G3322 2× SYBR Green qPCR Master Mix (Wuhan Seville Biotechnology Co., Ltd., China).

2.2 Preparation of C-AeP-P

Abelmoschus esculentus (okra) pods were prepared after removing seeds, cutting into sections, drying at 60 °C and crushing. Extract polysaccharide from dried pods by water extraction and alcohol precipitation, add 1/4 volume of sevage (Trichloromethane: N-Butanol = 1:1, v/v), shake violently and stand still, wait for protein coagulation and precipitation, and remove protein after layering with a separating funnel; Until the polysaccharide solution has no absorption peak at 280 nm in the full wavelength (190-400 nm) scanning spectrum, and it proves that the protein has been removed. After the residual sevage reagent was removed by spin evaporation and freeze-dried, the polysaccharide from *Abelmoschus esculentus (okra)* pod was obtained, named C-AeP-P.

2.3 Detection of rheological properties of C-AeP-P

Detection of static rheological properties of C-AeP-P

Accurately weighed C-AeP-P polysaccharide is dissolved in deionized water to prepare C-AeP-P solution (0.2%, 0.4%, 0.6%, 0.8%, 1.0% w/v). The apparent viscosities were tested with a steady-shear rate ranging from 0 to 100 s^{-1} at 25 °C using a Rheometer Mars 60 (Haag, Germany) equipped with a Cone plate geometry system (C35/1° Ti, gap size 1 mm).

Detection of dynamic rheological C-AeP-P

Five C-AeP-P solutions were dissolved at the concentration of 0.2-1.0% in distilled water with continuous stirring. The oscillatory dynamic tests were carried out on a rheometer (fixed oscillation strain 1%, angular frequency 0-10 Hz, 20 °C) and the storage modulus (G′) and loss modulus (G″) of C-AeP-P solutions were investigated.

2.4 Detection of rheological change of C-AeP-P solution by Ca^{2+} salt ion

C-AeP-P basic solution (1.2%) was prepared, and then $CaCl_2$ solution was added in equal volume. The final salt concentration were 0.10 M, 0.25 M and 0.50 M, and the final C-AeP-P solution concentration is based on the optimal results obtained in **Detection of dynamic rheological C-AeP-P**. The rheological properties were tested as described in the previous method.

2.5 Detection of C-AeP-P treatment on post-harvest quality control of Annona squamosa

The *A. squamosa* was treated with 0.6% concentration of C-AeP-P, C-AeP-P+Ca^{2+}, chitosan and pullulan polysaccharide respectively. The parameters were measured on the 2nd, 4th, 6th, 8th and 10th days of storage.

Soluble solids

Soluble solids test mainly refers to the experimental method of Zhang et al. (2022) and slightly modified (Zhang et al., 2022). The *A. squamosa* juice was collected by extrusion method, and the soluble solids were measured by hand-held refractometer HBR32 (Nanjing Haina Instrument Equipment Co., Ltd., China) at 20 °C, and the records were calculated as a percentage.

Firmness

The firmness was measured by GY-4 hand-held firmness tester (Yueqing adebao Instrument Co., Ltd., China). Repeat 3 times at different positions and take the average value.

Weight loss rate

The weight loss rate was calculated according to the following Formula 1:

$$\text{Weight loss rate}(\%) = \frac{W0\ (g) - W1\ (g)}{W0\ (g)} \times 100\% \quad (1)$$

W_0 is the weight of fruit before storage; W_1 is the weight of fruit after storage.

Surface color

The experimental assay was performed with a slight modification referring to the method of (Leal et al., 2022). The color (i.e. L^* value: brightness; a^* value: redness; b^* value: yellowness) was measured by portable color difference instrument WR-18 (Shenzhen Weifu Photoelectric Technology Co., Ltd., China). Seven places on the surface of *A. squamosa* were randomly selected for detection, and the results were taken as the average value. Calibrate with a whiteboard before testing.

Detection of glucose metabolizing enzyme gene expression

Extract *A. squamosa* RNA according to the steps of R2070 plant RNA small extraction kit. The operation of reverse transcription reaction as follows: Take a sterile enzyme free PCR tube and add 2 μg RNA solution; Add 1 μL Oligo (dT) 18 Primer (100 μM) and 1 μL Random hexamer primer, supplemented to 20 μL with deionized water without ribonuclease; Heat preservation on PCR instrument at 25 °C for 3 min; Continue adding 4 μL 5×

Reaction buffer and 1 μL service bio RT enzyme mix, mix well; Heat preservation on PCR instrument at 42 °C for 30 min; After that, the reverse transcriptase was inactivated at 85 °C for 1.3 min.

The PCR primer design was shown in Table 1. The RT qPCR reaction system was prepared as follows: The PCR mixtures contain 2× SYBR Green qPCR Master Mix (High ROX) 10 μL, forward primer (10 μM) 0.4 μL, reverse primer (10 μM) 0.4 μL and finally nuclease free water up to 20 μL. The PCR amplification was performed as follows: initial denaturation at 95 °C for 10 min, followed by 45 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 60 s, and single extension at 60 °C for 5 s, and end at 4 °C.

3 Results and discussion

3.1 Rheological properties of C-AeP-P

Static rheological properties

The static rheological properties of C-AeP-P concentrations subjected to steady shear in the range of 0.01-100 1/s (Figure 1). The results showed a sharp decrease in viscosity as shear rate increased, indicating the static rheological behavior of the C-AeP-P. Similarly, it shows that C-AeP-P solution has shear thinning characteristics. With the increase of shear rate, the binding between chains decreases, so the polysaccharide solution has shear thinning characteristics, which is also proved by relevant literature (Niu et al., 2018). Figure 1 also shows the viscosity of C-AeP-P is dependent on its concentration. With the increase of C-AeP-P concentration, the viscosity of the system increased. The apparent viscosity of C-AeP-P with different concentrations was fitted to Ostwald-de Waele Equation $\eta = K\gamma^{n-1}$ indicates a pseudoplastic characteristic when n is less than 1 (Xu et al., 2016). Take logarithms on both sides of the formula ($\eta = K\gamma^{n-1}$) to obtain linear equation of $\ln\eta = (n-1)\ln\gamma + \ln K$. As shown in Figure 2, R2 of each trend line is confirmed to reach 0.99. It indicates that the viscosity predicted by the model and the viscosity measured by the experiment show a high correlation coefficient, which is suitable for the sample. The n value is calculated according to the fitting curve equation, from the concentration of 0.2% to 1.0%, and the n values are 0.243, 0.332, 0.346, 0.385 and 0.397, respectively. If the viscosity remains constant with the increase of the shear rate of the test sample, the flow behavior index n is almost equal to 1 (ie, n ≅ 1), indicating that the sample have Newtonian behavior; otherwise, the test sample has non-Newtonian behavior (Camelo-Silva et al., 2022). It shows that C-AeP-P is a substance with obvious pseudoplastic fluid characteristics and non-Newtonian behavior. When the shear rate continuously increases from 0 to a fixed value, and then gradually decreases from this fixed value to 0, measures the change of solution shear stress with the shear rate, and takes the closed curve made by the shear stress as the Y-axis and the shear rate as the X-axis as the thixotropic ring. The larger the area of thixotropic ring, the greater thixotropy, and vice versa. The thixotropic rings are used to represent the energy required to destroy the internal structure of the solution. As shown in Figure 3, the thixotropic ring areas of C-AeP-P solution (0.2-1.0%) are 3.43, 3.51, 3.57, 4.75 and 7.96 respectively. With the increase of C-AeP-P concentration, the thixotropic ring area also increases.

Dynamic rheological properties

G′ represents the ability of polymer solution to resist deformation (i.e. solid like properties.). G″ represents the viscosity

Table 1. Primers of PCR.

Gene name	Primer sequence
AI	Forward primer: AGGAGGGAGGGCTGATTA
	Reverse primer: GGATTGACCCAAGCAAGAT
NI	Forward primer: TCAGGTGTTTGTCCGAGATT
	Reverse primer: CCCTTGAAGGTAGAGGGTC
SS	Forward primer: TATTGGGTCTACCCGACAC
	Reverse primer: CCAGCCCTTGACTCTTTAT
SPS	Forward primer: GGCTGAAGAATTGTCGCT
	Reverse primer: CTCAAGTTTCACATCGAAACC
GAPDH	Forward primer: GTGAGACTGGTGCTGAATATG
	Reverse primer: TGCTGGGAGCAGAAATAATAAC

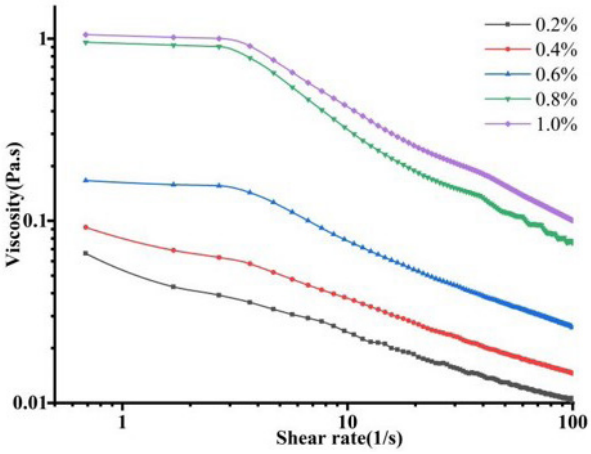

Figure 1. Effect of different concentrations on apparent viscosity of C-AeP-P.

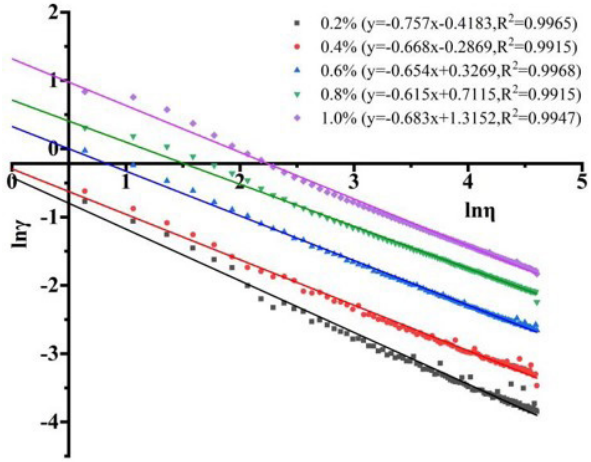

Figure 2. Curve of C-AeP-P with different concentrations.

characteristics of polymer solution (i.e. liquid like properties.). When tanδ = G″/G′ > 1, the solution shows more liquid like properties; On the contrary, the solution shows solid like properties (Mohamed et al., 2022). Figure 4 shows the effect of solution concentration on frequency sweep of C-AeP-P. With the increase of C-AeP-P concentration, G′ and G″ also increase (i.e. elasticity and viscosity increase with the increase of concentration.). When the C-AeP-P solution concentration is lower than 0.6%, tanδ > 1, the liquid like characteristics of C-AeP-P is more prominent; And the concentration is higher than 0.8%, tanδ < 1, the solid like characteristics of C-AeP-P are more prominent. Therefore, the concentration of 0.6% was selected as a follow-up study on the influence of salt ions on the rheology of C-AeP-P solution.

3.2 Effect of Ca^{2+} on rheological properties of C-AeP-P

Effect of Ca^{2+} on static rheological properties

As shown in Figure 5A-5D, Ca^{2+} is added with 0.6% C-AeP-P solution, which can effectively increase the apparent viscosity.

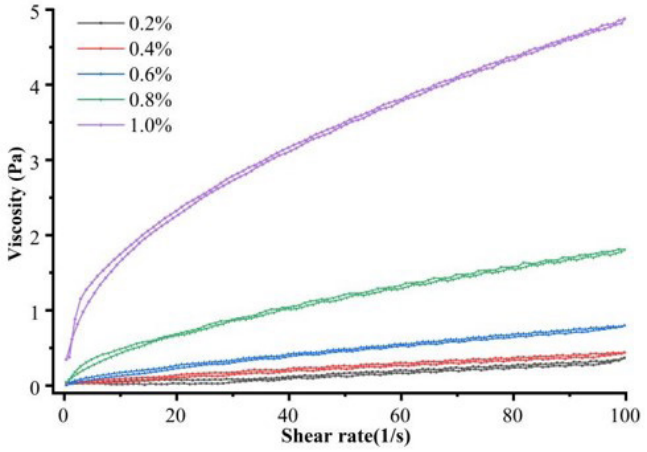

Figure 3. Thixotropic rings of C-AeP-P with different concentrations.

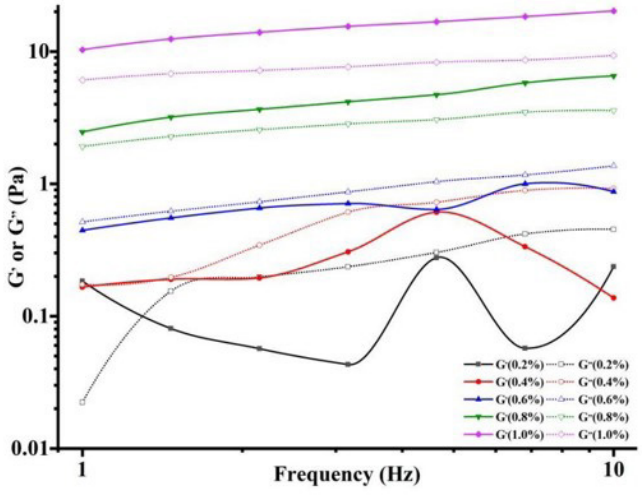

Figure 4. Strain sweep dependency of storage modulus (G′) and loss modulus (G″) for C-AeP-P at different concentrations.

It can also be pointed out that the increase of the apparent viscosity of the solution is dependent on the concentration of Ca^{2+}. With the increase of Ca^{2+} concentration, the apparent viscosity of the solution also increases, but the range is not high.

The addition of Ca^{2+} increases the area of thixotropic ring of 0.6% C-AeP-P solution (i.e. the energy required to destroy the internal structure of the solution increases with the addition of Ca^{2+}.) (Figure 6A-6D). With the increase of Ca^{2+} concentration (0.10, 0.25 and 0.50 M), the thixotropic ring area first increased and then decreased. The thixotropic ring areas of solution were 48.01, 49.63 and 47.47 respectively.

Effect of Ca^{2+} on dynamic rheological properties

With the increase of Ca^{2+} concentration, the G′ and G″ of C-AeP-P increased, and the solid like property of the solution increased gradually (Figure 7A-7D). When tanδ = 1, the strain scanning frequency is basically unchanged with the increase of Ca^{2+} concentration (i.e. Ca^{2+} in the selected concentration range basically does not affect the corresponding strain scanning frequency when tanδ = 1).

In conclusion, adding Ca^{2+} to 0.6% C-AeP-P solution can improve the viscosity, stability and solid like properties of the solution system. In the previous study, it was found that Ca^{2+} had a positive effect on the key enzymes of sugar metabolism in postharvest *A. squamosa*. Therefore, the quality control experiment of postharvest *Annona squamosa* was carried out by using C-AeP-P solution (0.6%) and adding calcium salt ion solution (0.1 M).

3.3 Change of C-AeP-P treatment Annona squamosa in post-harvest

Change of soluble solids

The change of soluble solids is used to evaluate the sugar content in fruits; And the change of sugar content in fruit is closely related to the maturity, the consume of sugar during respiration, the softening of cell wall and the change of fruit quality (Aday et al., 2013; Jiang et al., 2019; Nguyen et al., 2020). The changes of soluble solids in post-harvest *A. squamosa* are shown in Figure 8. In general, the soluble solids content of post-harvest treatment group and control group showed a upward trend during storage. The content of solids content in control group increased from 17.20 ± 0.59% to 31.40 ± 0.25%, the pullulan polysaccharide group increased from 17.08 ± 0.51% to 34.07 ± 0.58%, the chitosan group increased from 17.19 ± 0.55% to 27.20 ± 0.82% and then decreased to 19.57 ± 0.67%, the C-AeP-P group increased from 17.23 ± 0.42% to 32.10 ± 0.51%, and the C-AeP-P+Ca^{2+} group increased from 17.13 ± 0.52% to 30.10 ± 0.40%. It can be seen from the above results that the soluble solid content of *A. squamosa* treated with C-AeP-P and C-AeP-P+Ca^{2+} were lower than that of the control group on the 3rd-8th day of storage, especially on the 4th-6th day. However, with the increase of storage time, the gap gradually narrowed. On the 8th day, except for chitosan group, the soluble solids in other treatment groups reached the same level as the control group. By the 10th day, the soluble solids of pullulan polysaccharide

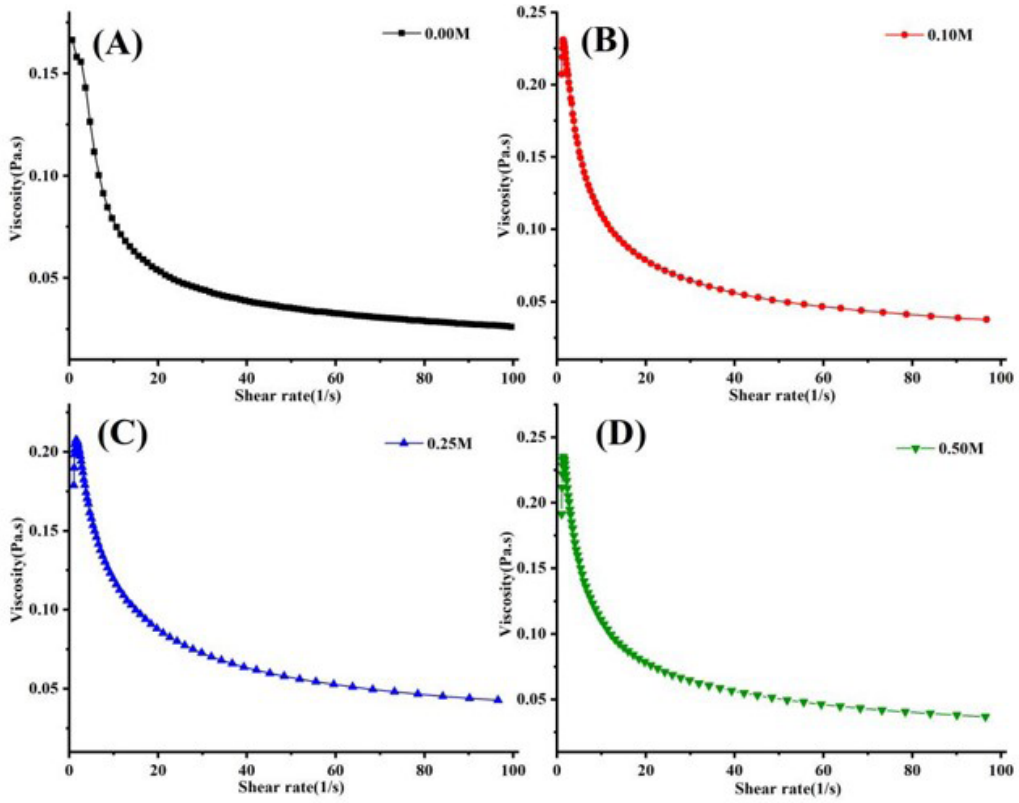

Figure 5. Effect of different Ca2+ concentrations ((A): 0.00M, (B): 0.10 M, (C): 0.25 M and (D): 0.50 M) on apparent viscosity of C-AeP-P (0.6%).

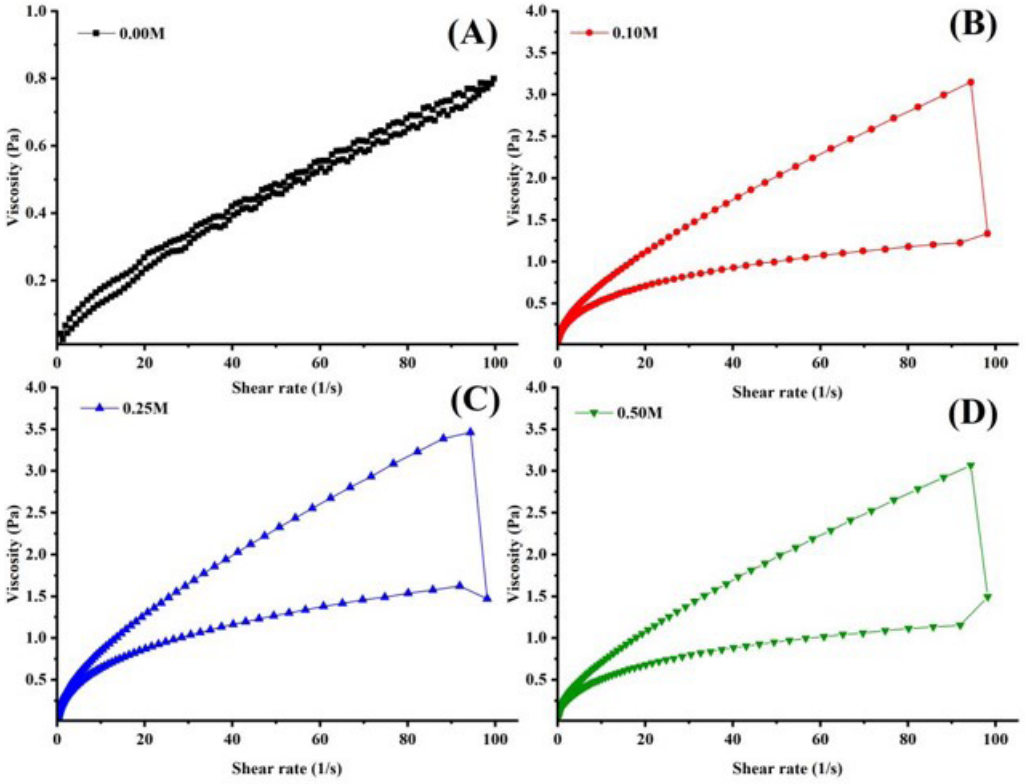

Figure 6. Thixotropic rings of C-AeP-P (0.6%) with different Ca2+ concentrations ((A): 0.00M, (B): 0.10 M, (C): 0.25 M and (D): 0.50 M).

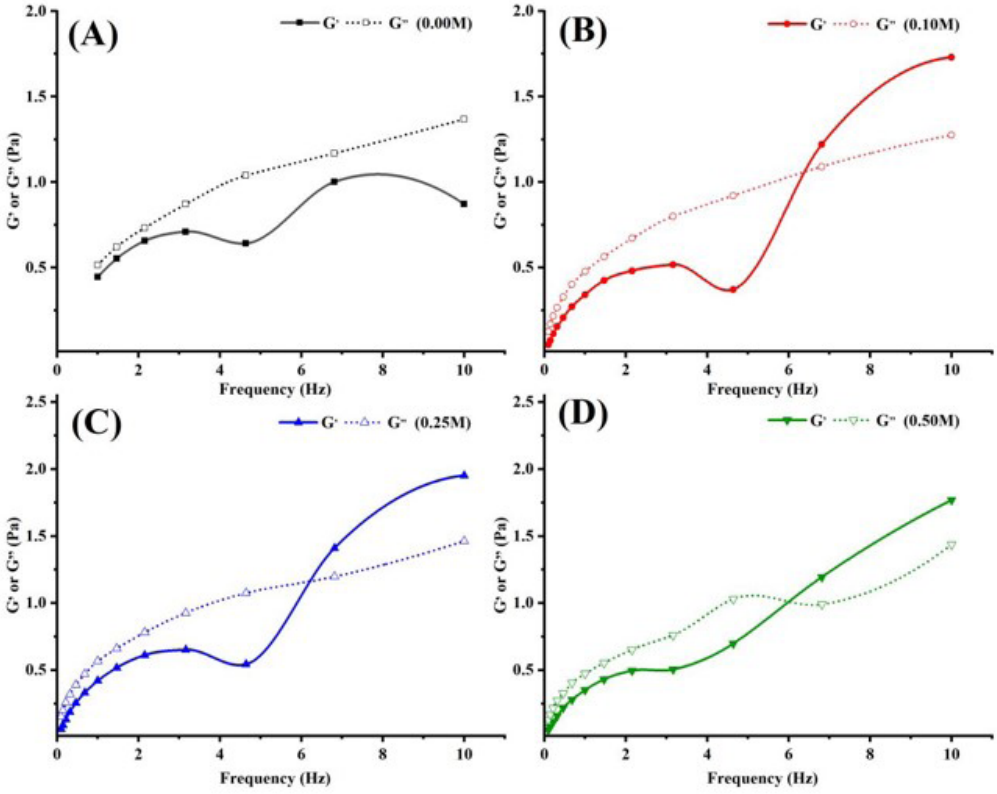

Figure 7. Strain sweep dependency of G′ and G″ for C-AeP-P (0.6%) at different Ca2+ concentrations ((A): 0.00M, (B): 0.10 M, (C): 0.25 M and (D): 0.50 M).

group had significantly exceeded that of the control group. The above results show that comparing the regulatory effects of chitosan and pullulan polysaccharide, C-AeP-P and C-AeP-P+Ca^{2+} are slightly better than pullulan polysaccharide on the whole, but weaker than chitosan.

Change of firmness

Firmness is one of the important indexes reflecting the changes of fruit storage tolerance and texture. During storage time, the firmness showed a downward trend (Figure 9). On the 4th day, the fruit firmness of C-AeP-P and C-AeP-P+Ca^{2+} treatment groups were significantly higher than control group. Until the 10th day, the firmness of C-AeP-P+Ca^{2+} group was better than the other groups. It also indicates that C-AeP-P can maintain the firmness of *A. squamosa*, and the addition of Ca^{2+} can promote the firmness maintenance effect.

Change of weight loss rate

Fruit weight loss is mainly caused by water loss during storage. The weight loss rate can prove the ability of storage methods to inhibit fruit water loss. The weight loss rate of *A. squamosa* in each group showed an upward trend during storage (Figure 10). On the 10th day, the chitosan group obtained the highest weight loss rate. However, the C-AeP-P treatment group were significantly smaller than that of the control group, indicating that C-AeP-P treatment can significantly reduce the weight loss

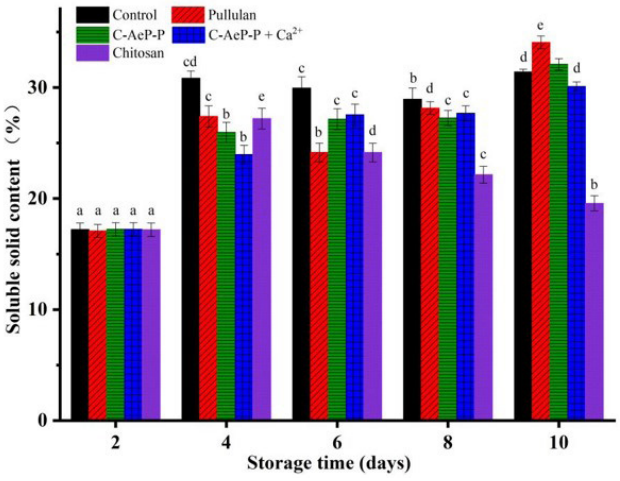

Figure 8. Changes of soluble solid content with storage time in different post-harvest treatments ("a-e" means the significant difference of the same treatment group in different storage time; $p < 0.05$.).

of *A. squamosa*; In addition, the addition of Ca^{2+} can promote the effect of C-AeP-P on reducing the weight loss rate.

Change of surface color

It can be seen from Figure 11 that the color difference L^* value of all groups decreased gradually during storage, indicating

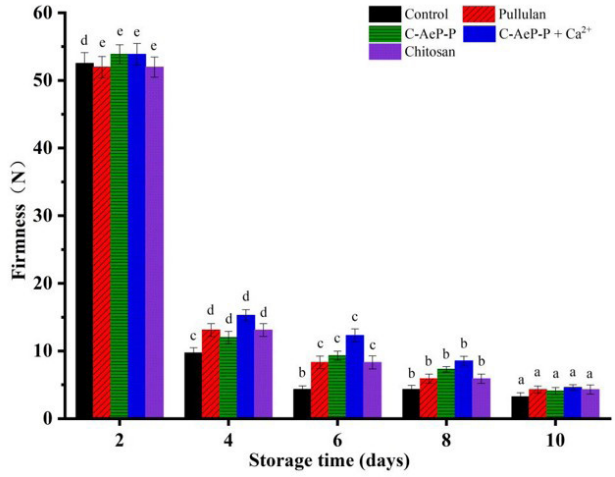

Figure 9. Changes of firmness with storage time in different post-harvest treatments ("a-e" means the significant difference of the same treatment group in different storage time; $p < 0.05$.).

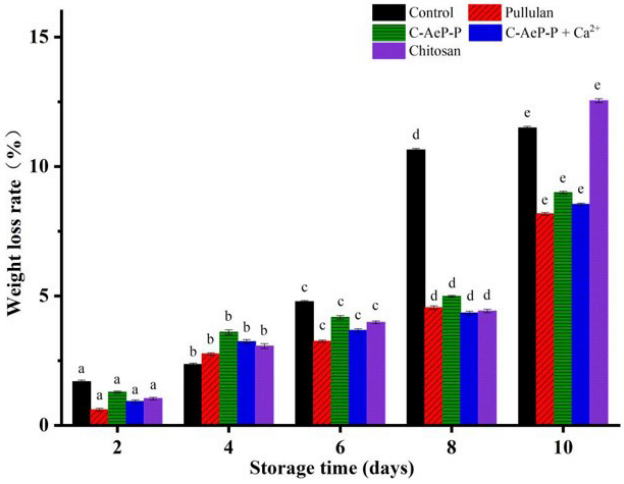

Figure 10. Changes of weight loss rate with storage time in different post-harvest treatments ("a-e" means the significant difference of the same treatment group in different storage time; $p < 0.05$.).

that the brightness of *A. squamosa* gradually darkened; The a^* value showed an upward trend, indicating that the fruit color gradually turned red; The b^* value decreased gradually, indicating that the fruit color gradually turned yellow. The brightness trend of C-AeP-P and C-AeP-P+Ca^{2+} treatment groups were always higher than the control group, but the redness was always lower than control group; On the 3rd-8th day, the yellowness trend of C-AeP-P and C-AeP-P+Ca^{2+} were higher than control group, especially on the 6th day, the difference between the treatment groups reached the maximum (The b^* value of chitosan group reached the maximum). Combined with the above results, C-AeP-P can maintain the color stability of *A. squamosa*, which is mainly reflected in the promotion of brightness and yellowness; In addition, the addition of Ca^{2+} can enhance the color retention function.

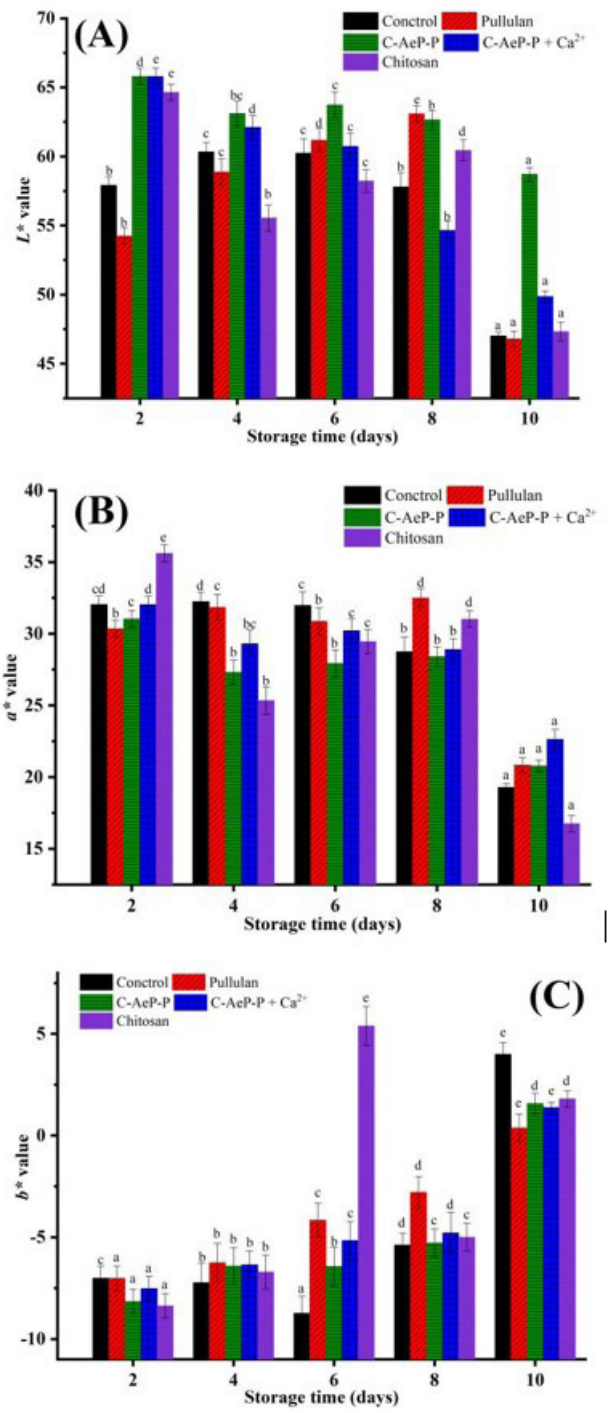

Figure 11. Changes of L^* value (A), a^* value (B) and b^* value (C) with storage time in different post-harvest treatments ("a-e" means the significant difference of the same treatment group in different storage time; $p < 0.05$.).

Change of glucose metabolizing enzyme gene expression

Sugar content is not only one of the key indicators of fruit nutrition, but also can be used to investigate the changes of fruit quality during storage. Fruit softening is related to the degradation of cell wall substances, such as pectin, cellulose and hemicellulose and

other sugar substances, mainly caused by changes in the activity of hydrolytic enzymes (Qu et al., 2022). However, enzyme production is regulated by genes. A very obvious feature of *Annona squamosa* during storage is sugar metabolism and accumulation. As shown in the following Table 2, in our lab previous research work, we found that acid invertase (*AI*), neutral invertase (*Ni*), sucrose synthase (*SS*) and sucrose phosphate synthase (*SPS*) in *A. squamosa* fruit are closely related to glucose metabolism (Ren et al., 2019). Therefore, in the following experiments, we will investigate the effect of C-AeP-P on the gene expression of sugar metabolizing enzyme in *A. squamosa* during storage, so as to clarify its regulation effect on the quality.

Figure 12 shows the expression of four genes of glucose metabolizing enzymes (*AI*, *Ni*, *SS* and *SPS*) in C-AeP-P treated *A. squamosa* during storage.

Acid invertase and neutral invertase are two important sucrose catabolic enzymes that affect sugar metabolism during post-harvest storage; Invertase can catalyze the conversion of sucrose into fructose and glucose; Moreover, the changes of invertase activity of different kinds of fruits during post-harvest storage are also different (Cao et al., 2013; Zhu et al., 2013). As shown in Figure 12A, the expression of *AI* gene increases with the extension of storage time. It is also known from the figure

Table 2. Correlation between soluble sugar and glucose metabolizing enzyme gene expression in *Annona squamosa* during storage.

	Soluble sugar			Glucose metabolizing enzyme gene			
	Fructose	Glucose	Sucrose	*AI*	*NI*	*SS*	*SPS*
Fructose	1						
Glucose	0.944**	1					
Sucrose	-0.358	-0.356	1				
AI	0.271	0.310	-0.841**	1			
NI	-0.186	-0.026	0.551*	-0.437	1		
SS	-0.132	-0.306	-0.101	-0.145	-0.769**	1	
SPS	-0.569*	-0.616**	0.708**	-0.531*	0.095	0.368	1

Tthe correlation type is Pearson type. *Significant correlation ($p < 0.05$). **Extremely significant correlation ($p < 0.01$).

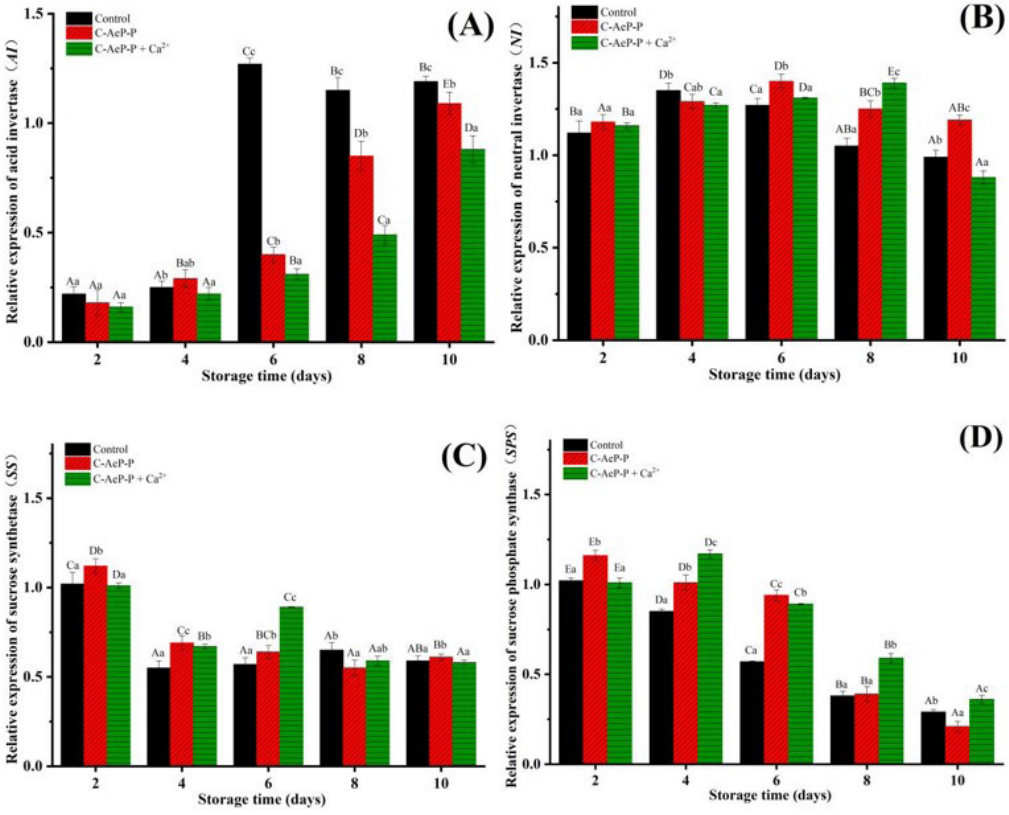

Figure 12. Changes of *AI* (A), *NI* (B), *SS* (C) and *SPS* (D) gene expression with storage time in different post-harvest treatments ("A-E" means the significant difference of the same treatment group in different storage time; "a-c" means the significant difference of the different treatment groups in the same storage time; $p < 0.05$.).

that the addition of Ca^{2+} can strengthen this regulation. On the 6th day, when the *AI* expression in the control group reached the highest, the C-AeP-P and C-AeP-P+Ca^{2+} treatment groups were still at a low level; Compared with the control group, they were 31.50% and 24.41% of the control group, respectively. During the whole storage period, the expression of *AI* in C-AeP-P and C-AeP-P+Ca^{2+} treatment groups was always lower than control group. In previous studies, it has been proved that there is a very significant correlation between sucrose and *AI* gene expression during *A. squamosa* storage. Therefore, it can be considered that C-AeP-P and C-AeP-P+Ca^{2+} can regulate sucrose during *A. squamosa* storage. *Ni* gene expression increased slowly and then decreased rapidly (as shown in Figure 12B); The control group, C-AeP-P and C-AeP-P+Ca^{2+} treatment group reached the highest and began to decline on the 4th, 6th and 8th day of storage, respectively. C-AeP-P treatment significantly prolonged the time of reaching the maximum expression of *Ni*, and t.he additional addition of Ca^{2+} enhanced the regulation ability of C-AeP-P.

Sucrose synthetase and sucrose phosphate synthase also play important role in the process of fruit sugar accumulation and formation, which is very important for the quality of postharvest fruit (Wang et al., 2019). There is a close relationship between enzyme activity and gene expression. As shown in Figure 12C, the expression of SS gene generally showed downward trend with the extension of storage time. The control group, C-AeP-P and C-AeP-P+Ca^{2+} treatment group decreased to the lowest level on the 4th, 8th and 8th days respectively. C-AeP-P treatment can delay the decline rate of SS expression. Figure 12D shows that C-AeP-P treatment can delay the decline rate of SPS gene expression, especially the regulation effect of C-AeP-P+Ca^{2+} on SPS gene is significantly enhanced.

In conclusion, C-AeP-P can delay the changes of gene expression of key enzymes of glucose metabolism during storage of *A. squamosa*, especially Ai and Ni gene expression. The effect of C-AeP-P is mainly reflected in delaying glucose metabolism and making the fruit closer to the initial storage state; In addition, C-AeP-P can regulate and maintain the quality of *A. squamosa*. The addition of Ca^{2+} can strengthen this regulation. This phenomenon may be attributed to the fact that Ca^{2+} increases the viscosity of C-AeP-P and better adheres to *A. squamosa*, which makes Ca^{2+} play a better regulatory role; Or Ca^{2+} and C-AeP-P have a synergistic effect, which enhances the regulation of sugar metabolizing enzyme gene expression in *A. squamosa*. However, the above conjecture needs further experimental verification.

4 Conclusion

In this study, the rheological properties of C-AeP-P solution showed the properties of typical non-Newtonian fluids. C-AeP-P shows the characteristics of shear thinning. With the increase of the mass percentage of C-AeP-P, the apparent viscosity and non-Newtonian coefficient increase; The tangent of loss angle decreases gradually, and the performance of solid like is more prominent. The addition of Ca^{2+} can effectively increase the apparent viscosity of C-AeP-P solution. Similarly, the addition of calcium salt ions can also increase the area of thixotropic ring of C-AeP-P solution. The rheology of C-AeP-P solution is affected by both salt ion and concentration. The Ca^{2+} can not only improve the viscosity and stability of C-AeP-P solution, but also significantly affect the quality and sugar metabolism enzyme activity of *A. squamosa*. The treatment of C-AeP-P and C-AeP-P+Ca^{2+} can effectively slow down the quality changes of *A. squamosa* during storage, such as the rapidly decline of fruit hardness, the rapidly increase of soluble solid content, the highly weight loss rate, the largely color change and so on. At the same time, C-AeP-P can regulate the expression of key enzyme genes *AI*, and *Ni* of sugar metabolism in *A. squamosa* during storage, so as to delay the sugar metabolism during storage and regulate the quality of *A. squamosa*; The addition of Ca^{2+} enhanced the regulation effect of key enzyme gene expression. In general, the treatment of *A. squamosa* fruit with C-AeP-P and C-AeP-P+Ca^{2+} during storage provides help to solve the problems of fruit softening, short maturity, poor storage, serious water loss and large color difference.

Conflict of interest

There are no conflicts of interest declared by the authors.

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